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ORIGINAL PAPER

# Characterization of new loci for Hessian fly resistance in common wheat

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**Abstract** The discovery of several new loci for resistance to Hessian fly was reported here. *QHf.uga-6AL*, the late *HR61* was recognized from wheat cultivar 26R61 on the distal end of 6AL with resistance to both biotypes E and *vH13*. It is the first gene or QTL found on this particular chromosome. *QHf.uga-3DL* and *QHf.uga-1AL*, physically assigned to the deletion bins 3DL2-0.27–0.81 and 1AL1-0.17–0.61, respectively, were detected for resistance to biotype *vH13*. Both QTL should represent new loci for Hessian fly resistance and the latter was detectable only in the late seedling stage when tolerance was evident. In addition, *QHf.uga-6DS-C* and *QHf.uga-1AS* had minor effect and were identified from the susceptible parent AGS 2000 for resistance to biotype E and *vH13*, respectively. *QHf.uga-6DS-C* is different from the known gene *H13* on 6DS and *QHf.uga-1AS* is different from *H9* gene cluster on 1AS. These loci also might be new components of Hessian fly resistance, although their LOD values were not highly significant. The QTL detections were all conducted on a RIL mapping population of 26R61/AGS 2000 with good genome coverage of molecular markers. The strategy used in the current study will serve as a good starting point for the discovery and mapping of resistance genes including tolerance to the pest and the closely linked markers will certainly be useful in selecting or pyramiding of these loci in breeding programs.

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**Abbreviations**

| | |
|---|---|
| DArT | Diversity arrays technology |
| LOD | Logarithm of odds |
| QTL | Quantitative trait locus (loci) |
| RIL | Recombinant inbred line |
| SSR | Simple sequence repeat (microsatellite) |
| 1AS | The short arm of chromosome 1A |
| 1AL | The long arm of chromosome 1A |
| 2AS | The short arm of chromosome 2A |
| 3DL | The long arm of chromosome 3D |
| 6AL | The long arm of chromosome 6A |
| 6DS | The short arm of chromosome 6D |
| 6DS-C | The short arm of chromosome 6D near centromere |

## Introduction

The Hessian fly, *Mayetiola destructor* (Say), which is believed to have originated from west Asia in the Fertile Crescent, is one of the most destructive insect pests of common wheat (*Triticum aestivum* L.) (Barnes 1956; El Bouhssini et al. 2009; Harris et al. 2003). It was first found on Long Island, New York in the USA in 1770s, and subsequently spread southward and westward, to most of the wheat-growing regions of the nation (Packard 1880, 1928; Rockwood and Reeher 1933). Historically, at least six periods of serious damage are recognized in 1779 (only in New York), 1790–1792, 1817, 1844–1846, 1871–1872, and 1876–1877 with irregular intervals (Packard 1880). The period of 1876–1877 was the most devastating outbreak in which at least 14 states were heavily infested leading to crop failure with plants being either totally or partially destroyed. From the year 1900 afterwards, the Hessian fly remained one of the most important pests in the USA particularly in the Southeast (Buntin and Chapin 1990; Cambron et al. 2010; Morton et al. 2011). The losses due to Hessian fly damage in the state of Georgia alone were estimated at $28 million in a single year of 1989 (Hudson et al. 1991).

Currently, the recommended control of Hessian fly is via an integrated pest management (IPM) approach that may include cultural control, chemical control, biological control, and host–plant resistance also called genetic control (Buntin et al. 1992; Porter et al. 2009). Host–plant resistance is of extreme importance and serves as foundation of a successful IPM strategy. Thus far, 32 genes designated *H1* through *H32* have been discovered within wheat and from related species (McIntosh et al. 2008). Of these genes, 14 were identified from *T. turgidum* ssp. *durum*, eight from common wheat, six from *Aegilops tauschii*, two from rye, and the remaining two genes *H27* and *H30* were derived from *Ae. ventricosa* and *Ae. triuncialis*, respectively (Delibes et al. 1997; Martín-Sánchez et al. 2003). In commercial wheat production, three genes *H3*, *H6*, and *H5*, initially deployed in wheat cultivars 'Dual' in 1955 (Caldwell et al. 1957), 'Knox 62' in 1962 (Patterson et al. 1978), and 'Arthur 71' in 1971 (Patterson et al. 1975), respectively, have had a proven track record of reduction in infestation levels of Hessian fly in the eastern soft red winter wheat region (Foster et al. 1991). Other genes, such as *H9*, *H13*, *H21*, *H25*, *H26* etc., have been added or are presently being added into diverse wheat germplasm in the USA (Cainong et al. 2010; Cambron et al. 2010; Chen et al. 2009a; Johnson et al. 2009; Ratcliffe 2012). However, the resistance genes tend to breakdown when they are deployed in a large area and over a long time, since the growing of highly resistant cultivars exerts a strong selection pressure on Hessian fly population that favors virulent biotypes surviving and reproducing on resistant wheat, consequently posing great threat to the permanence of the resistance (Ratcliffe and Hatchett 1997).

New sources of Hessian fly resistance are therefore urgently necessary to incorporate into wheat breeding programs, especially in the southeastern region of the USA, where Hessian fly has the most diverse genetic variations and the greatest number of generations per year due to mild winter conditions (Buntin and Chapin 1990; Cambron et al. 2010; Porter et al. 2009; Ratcliffe et al. 1997, 2000). 'Pioneer® variety 26R61' (shorten as 26R61 hereafter), a check cultivar used in Uniform Southern Soft Red Winter Wheat Nursery (USSRWWN), has shown good resistance to Hessian fly biotype E at the seedling stage across different years (http://www.ars.usda.gov/main/docs.htm?docid=21894) and biotype *vH13*, a virulent biotype to *H13*. However, its resistance has not yet been clarified. The objectives of this research are to genetically characterize the QTL or genes for resistance to biotype E and *vH13* based on an RIL mapping population of 26R61/'AGS 2000' (AGS 2000 is susceptible to both biotypes), to determine their relationships with other known Hessian fly resistance genes, and to shed some light on the matter of tolerance to the injury by Hessian fly.

## Materials and methods

### Plant materials and Hessian fly biotypes

A RIL population of 178 $F_{6:7}$ lines developed from a cross between soft red winter wheat cultivars 26R61 (PI 612153) and AGS 2000 (PI 612956) by single-seed descent was used. The cultivar 26R61 (Omega 78/S76/Arthur 71/3/Stadler//Redcoat/Wisconsin 1/5/Coker 747/6/PIO2555 sib) was developed by Pioneer Hi-Bred, and AGS 2000 (PIO2555/PF84301//FL302) was developed and released jointly by the University of Georgia and University of Florida in 1999 (Johnson et al. 2002). The population (abbreviated as PR61/A2000) was reported by Hao et al. (2011, 2012) for genetic studies of wheat stripe rust and *Soil-borne wheat mosaic virus* resistance.

Three Hessian fly biotypes designated E, L, and *vH13* were selected for infesting parents, checks, and/or mapping population. The checks, including 'Blueboy' (no *R* gene), 'Newton' (no *R* gene), 'Carol' (*H3*), 'Caldwell' (*H6*), 'Seneca' (*H7H8*), 'Iris' (*H9*) and 'Molly' (*H13*) served as controls or as differentials for defining Hessian fly biotypes, and were added in specific tests with certain combinations (Cambron et al. 2010; Chen et al. 2009b; Patterson et al. 1994). All the Hessian fly biotypes were maintained in the USDA-ARS Crop Production and Pest Control Unit, Purdue University, West Lafayette, Indiana, USA.

### Hessian fly infestation and resistance evaluation

Initial screening of the parents, 26R61 and AGS 2000, against biotype E and L was conducted at two temperature regimes (16 and 24 °C). The two parents did not confer resistance to biotype L, and biotype E was finally chosen to infest the entire mapping population at the low temperature of 16 °C. The methods of infestation and evaluation were similar to those of Ratcliffe et al. (2002) and Cambron et al. (2010). Briefly, each flat (54 × 36 × 8 cm) was divided into two equal parts, the top half included 10 RILs of PR61/A2000 and the two parents, while the bottom half included another 10 lines and resistant/susceptible checks Cardwell (*H6*)/Carol (*H3*). The parents and checks were always placed in the middle of each flat. For each entry, about 20 seeds were evenly planted. As such, a total of nine flats for the entire mapping population of 178 RILs were planted and placed in a controlled growth chamber at 16 °C with a photoperiod of 14:8 h (light/dark) cycle.

When the seedlings were in the 1.5-leaf stage, with the second leaf starting to emerge, the flats were covered with cheesecloth tents and about 300 gravid females were immediately released inside for 4–5 days, after which the tent was removed. Plant response was recorded after 2–3 weeks. Plants were rated as resistant (R) if they exhibited a normal growth habit and contained dead first-instar larvae, and plants were rated as susceptible (S) when they showed stunting and a dark green color and contained living larvae. Plants with a normal green leaf color and normal standing, but without dead larvae were considered escapes and were discarded in calculation. The final data were recorded as the number of R and S plants for each entry and converted to percentage resistance for QTL analysis. The test described here included two independent experiments with the same procedure carried out in 2011 and 2012, respectively. The only difference between the experiments was the susceptible check in 2012 was Newton (no *R* gene) rather than Carol (*H3*).

Another biotype *vH13* was also used. The test followed the same procedures as the biotype E test. Differently, the temperature was set at 18 °C and the two parents were not included in each flat but only added once after the RILs in the last flat. The checks in the top half of each flat were Newton (no *R* gene) and Seneca (*H7H8*), and in the bottom were Iris (*H9*) and Molly (*H13*). In the test of the entire mapping population, the rating was taken twice as in early and late stage of the seedling development, respectively. In addition to ‘R’ and ‘S’ ratings, a third category ‘T’ (tolerance) was added in the late stage, because it was apparent in that some seedlings of some of the lines had been stunted, but were growing out of the injury with live larvae on the plant. We considered T as S in the early seedling stage but as R in the late seedling stage when converting the rating data to percentage resistance.

### Data analysis and QTL mapping

Sets of rating data were converted to percentage resistance in Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA). The SAS statistical package was used for basic statistic analysis and output of the histograms (SAS Institute, Cary, NC, USA). The genetic linkage maps used for QTL analysis were described by Hao et al. (2012) with updates of QTL target regions in the present study. Altogether, the maps include 984 loci on 25 linkage groups, with gaps for chromosomes 2A, 4D, 7A and 7D. The maps span 2,625 cM, with 1,068, 841, and 716 cM in the A, B, and D genomes, respectively. QTL detection was conducted in Windows QTL Cartographer 2.5 (Wang et al. 2012): composite interval mapping (CIM) method was used; walk speed was set as 1.0 cM and the control parameters were default; threshold of LOD (logarithm of odd) was set as 2.5. QTL designation referred to the guidelines for nomenclature of QTL in wheat (McIntosh et al. 1998).

## Results

### Reactions of the parental lines to biotype E and L

Both 26R61 and AGS 2000 were susceptible to biotype L at high (24 °C) and low (16 °C) temperature regimes (Table 1). For the biotype E test, AGS 2000 was always susceptible, whereas 26R61 was partially resistant (13R-12S) at the high temperature and completely resistant (26R-0S) at the low temperature (Table 1). The susceptible check Blueboy showed a completely susceptible response as expected across all the tests (Table 1). The results confirmed the previous rating data of the two parents in USSRWWN from 1998 to 2011 (http://www.ars.usda.gov/main/docs.htm?docid=21894) as shown in Supplementary Table S1: 26R61 was resistant to biotype E and O, and susceptible to biotype B, C, D, L in the seedling; and AGS 2000 was susceptible to all the biotypes used in the uniform nursery tests.

### Identification of QTL for resistance to biotype E

Since the resistance in 26R61 was fully expressed at low temperature of 16 °C, all the biotype E tests were conducted under this condition. Based on the rating data in the 2 years, 26R61 (157R-7S, 2011, 101R-3S, 2012) and the check cultivar Caldwell (114R-10S, 98R-5S) were rated as R, and AGS 2000 (0R-136S, 7R-76S) and the other two

**Table 1** Screening of parents and checks against Hessian fly biotypes E and L in controlled environments

| Cultivar | Bio L 24 °C | Bio L 16 °C | Bio E 24 °C | Bio E 16 °C | Bio E-2011 | Bio E-2012 |
|---|---|---|---|---|---|---|
| 26R61 (*HR61*, +) | 0–25[a] | 0–23 | 13–12 | 26-0 | 157–7[b] | 101–3[c] |
| AGS 2000 | 0–27 | 0–30 | 0–21 | 0–25 | 0–136 | 7–76 |
| Blueboy (none) | 0–17 | 0–21 | 0–28 | 0–22 | NA | NA |
| Carol (*H3*) | NA[d] | NA | NA | NA | 0–112 | NA |
| Caldwell (*H6*) | NA | NA | NA | NA | 114–10 | 98–5 |
| Newton (none) | NA | NA | NA | NA | NA | 0–124 |

[a] Rating was recorded as R-S, number of resistant plants versus number of susceptible plants

[b] Consensus data of R-S in 2011 biotype E test for parents or checks

[c] Consensus data of R-S in 2012 biotype E test for parents or checks

[d] Not applicable

checks Carol (0R-112S, 2011) and Newton (0R-124S, 2012) were categorized as S (Table 1). The rating data of these checks matched well with the reactions of differentials to biotype E (Supplementary Table S1). For the RILs, the distribution of the rating data deviated significantly from the normal distribution ($P < 0.01$) in all environments as shown in Fig. 1 (left three graphs).

A major QTL, *QHf.uga-6AL*, was stably detected from 26R61 in all three environments on the basis of the whole genome scanning and the CIM analysis (Supplementary Fig. S1; Table 2). The interval flanked by markers *Xgwm427* and *wPt-731936* was significant in all environments (Fig. 2), and explained up to 63 % of the mean phenotypic variation (Table 2). The peak LOD values of 26.0, 17.9 and 30.1 in 2011, 2012 and Mean, respectively, were all highly significant (Table 2). In addition, two QTL of minor effect designated *QHf.uga-2AS* and *QHf.uga-6DS-C* were also identified on 2AS and 6DS-C, respectively (Fig. 3; Table 2). *QHf.uga-2AS* from 26R61 was flanked by markers *Xbarc124* and *Xgwm359* (closer to *Xgwm359*) and *QHf.uga-6DS-C* from AGS 2000 was situated between *wPt-665166* and *Xgwm325* (Fig. 3). Both QTL were detected only in the environment of 'BioE-Mean' with suggestive LOD values and explained about 4 % of total phenotypic variation (Table 2).

### Identification of QTL for resistance to biotype *vH13*

For the two parents, 26R61 was rated as R (18R-0S-0T), and AGS 2000 was S (2R-17S-0T) when tested against biotype *vH13* at the temperature of 18 °C. Under the same condition, the check cultivars Seneca (*H7H8*) and Iris (*H9*) exhibited 107R-4S-3T and 100R-1S-0T, respectively, and were rated as R; whereas the other two checks Newton (none, 2R-140S-0T) and Molly (*H13*, 17R-135S-1T) were rated as S. For the RILs, similar to the biotype E test, the frequencies of the rating data deviated significantly from the normal distribution ($P < 0.01$) both in the early and the late stages as shown in Fig. 1 (right two graphs).

A total of three and five QTL were detected in the early and late stage, respectively, through the whole genome scanning and CIM analysis (Supplementary Fig. S2; Table 2). Interestingly, the same QTL of major effect *QHf.uga-6AL* as mentioned earlier was identified, which was closely linked with marker *Xgwm427*, and explained about 21 % of trait variation in the early stage, and 13 % in the late stage (Fig. 2; Table 2). Furthermore, a new QTL named *QHf.uga-3DL* was detected in both stages. It was situated between the markers *Xcfd4b* and *Xgwm52* on 3DL, and contributed about 9 and 11 % of phenotypic variations for each stage (Fig. 3; Table 2). Another QTL (*QHf.uga-1AL*) was detected only in the late stage, flanked by SSR markers *Xgwm135* and *Xcfa2129* on 1AL, which account for about 9 % of total phenotypic variation (Fig. 3; Table 2). These QTL all had highly significant LOD values at the peak positions and were derived from the resistant parent 26R61 (Table 2). In addition, *QHf.uga-2AS* in both stages and *QHf.uga-1AS* only in the late stage were detectable on 2AS and 1AS, respectively, but their LOD values were not highly significant (Table 2). *QHf.uga-2AS* from 26R61 was closely linked with marker *Xbarc124*, and *QHf.uga-1AS* from the susceptible parent AGS 2000 was flanked by DArT markers *wPt-665351* and *wPt-731617*. Both QTL explained about 6–7 % of trait variations (Fig. 3; Table 2).

## Discussion

In the present research, reactions of the wheat cultivars 26R61 and AGS 2000 to Hessian fly biotype E were confirmed, and the condition for the gene expression in 26R61 was also optimized (Table 1; Supplementary Table S1). *QHf.uga-6AL*, the major determinant of resistance to biotype E in PR61/A2000 population, was situated between markers *Xgwm427* and *wPt-731936* in the genetic map (Fig. 2). Because the proximal marker *Xgwm617*, the marker *Xgwm427* and the distal marker *wPt-7204* (situated between markers *wPt-731936* and *wPt-5654*) all located on the 10 % distal part of 6AL in physical maps (Francki et al. 2008; Sourdille et al. 2004), *QHf.uga-6AL* was further assigned to the deletion bin 6AL8-0.90–1.00. To the authors' knowledge, it is the first gene or QTL found on this particular chromosome of 6A in wheat. On the basis of a large contribution of the QTL to trait variation and the unique chromosome location, one new gene is therefore proposed in 26R61 for resistance to Hessian fly biotype E and temporarily designated *HR61*. It was noted the $R^2$

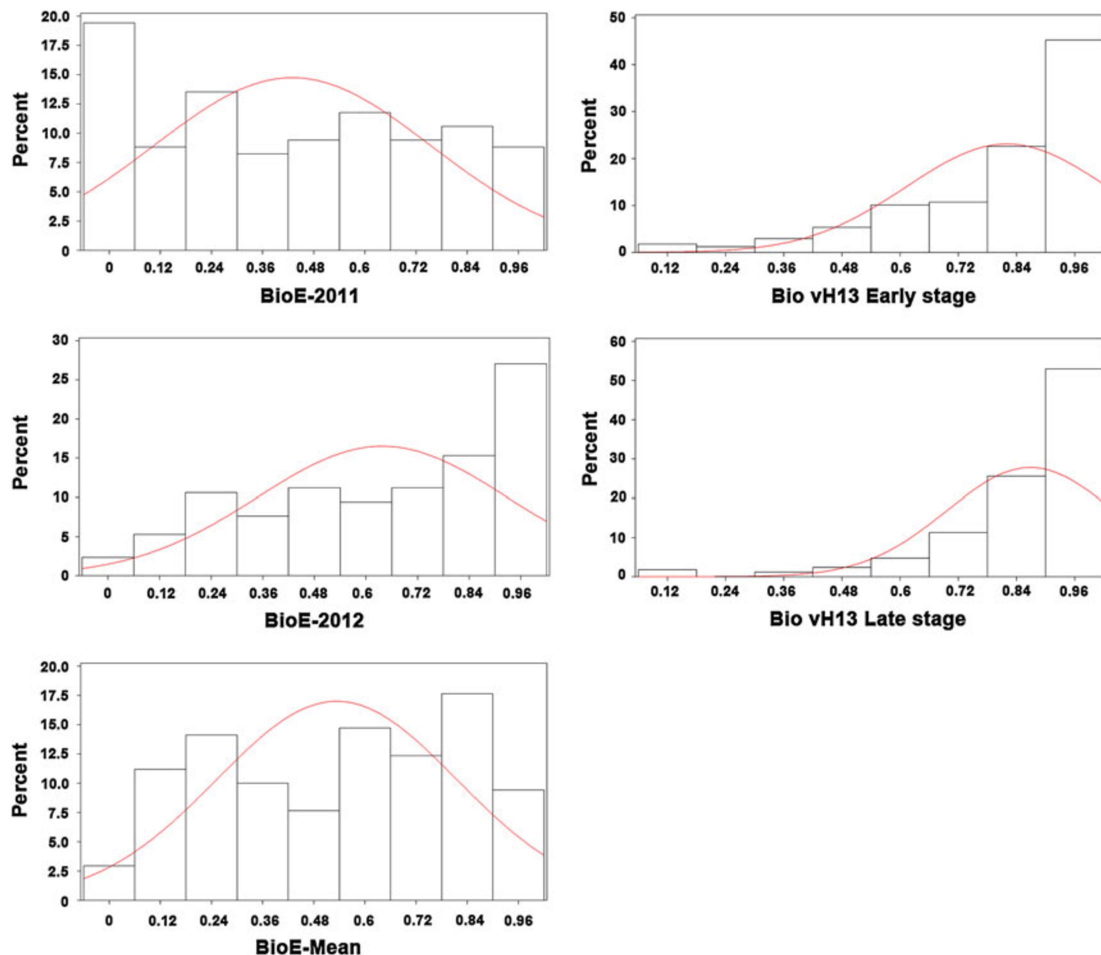


**Fig. 1** Histogram of rating data for biotype E test (*left three graphs*) in three environments (include the means) and biotype *vH13* test (*right two graphs*) in two seedling stages of PR61/A2000 population; the *curved lines* are the normal distribution curves

value in the environment of BioE-Mean (63 %) was higher than those in 'BioE-2011' (48 %) and in 'BioE-2012' (38 %) environments (Table 2), probably due to the poor infestations of some flats in both years, but fortunately the flats (RILs) with escapes were different between years (data not shown), which apparently lead to the mean values being more competitive over the data in individual years.

Surprisingly, 26R61 and Seneca (*H7H8*) had very similar reactions to different Hessian fly biotypes and were susceptible to biotype B, C, D and L, and resistant to biotype E, O and *vH13* (Supplementary Table S1). Seneca (CI 12529) firstly reported to have *H7H8* gene combination for resistance to biotype E (Patterson and Gallun 1973), and was used as one of the differentials for defining the original 16 Hessian fly biotypes (Gallun 1977). In Seneca *H7* was assigned to chromosome 5D and *H*8 to chromosome 2D or 7D (also possible on 2A or 6D). They exhibited complementary epistasis based on a strong evidence that resistant plants were recovered from a cross between two susceptible progenies, which meant that they must be paired for either gene to express resistance fully (Amri et al. 1990). However, for 26R61, only one gene was detected for resistance to biotype E in the present study. Based on the conflicts of chromosomal location and gene interaction, it appears that *HR61* should be different from *H7H8* in Seneca. In addition, our attention was drawn to another gene combination *H1H2* from common wheat cultivar 'Dawson' (Cartwright and Wiebe 1936; Noble and Suneson 1943). Dawson (CI 3342) was resistant to California Hessian fly population, but was susceptible to Indiana population in 1930s–1940s (Cartwright and Noble 1947). It was assumed the majority biotype was 'GP' (Great Plains) in California and was 'A' in Indiana at that time. *H1H2* was definitely susceptible to more virulent biotypes B, C, D and L in Indiana (Gallun 1977). However, in Georgia in the late 1980s, *H1H2* showed the same reaction as *H7H8* in Seneca, with both being highly resistant to field populations of Hessian fly consisting primarily of biotypes G, E, and O (Buntin et al. 1990). We therefore speculate that the gene combination *H1H2* is

**Table 2** Position and effect of Hessian fly resistance QTL across environments based on CIM analysis of a 26R61 × AGS 2000 cross

| Environment | QTL name | Interval | Peak LOD | Peak position (cM) | $R^2$ (%)[a] | Additive effect[b] |
|---|---|---|---|---|---|---|
| BioE-2011 | ***QHf.uga-6AL***[c] | *Xgwm427–wPt-731936* | 26.0** | 160.2 | 48 | 0.23 |
| BioE-2012 | ***QHf.uga-6AL*** | *Xgwm427–wPt-731936* | 17.9** | 160.2 | 38 | 0.19 |
| BioE-Mean | *QHf.uga-2AS* | *Xbarc124–Xgwm359* | 2.6 | 28.0 | 4 | 0.06 |
| | ***QHf.uga-6AL*** | *Xgwm427–wPt-731936* | 30.1** | 160.2 | 63 | 0.23 |
| | *QHf.uga-6DS-C* | *wPt-665166–Xgwm325* | 2.8 | 55.0 | 4 | −0.06 |
| Bio vH13 early | *QHf.uga-2AS* | *Xbarc124–Xgwm359* | 3.4 | 19.1 | 7 | 0.07 |
| | *QHf.uga-3DL*[d] | *Xcfd4b–Xgwm52* | 5.1** | 74.1 | 9 | 0.08 |
| | ***QHf.uga-6AL*** | *Xgwm427–wPt-731936* | 9.5** | 159.2 | 21 | 0.12 |
| Bio vH13 late | *QHf.uga-1AS* | *wPt-665351–wPt-731617* | 2.7 | 30.7 | 7 | −0.07 |
| | *QHf.uga-1AL*[e] | *Xgwm135–Xcfa2129* | 4.6** | 61.2 | 9 | 0.08 |
| | *QHf.uga-2AS* | *Xbarc124–Xgwm359* | 3.0 | 17.0 | 6 | 0.06 |
| | *QHf.uga-3DL* | *Xcfd4b–Xgwm52* | 5.8** | 75.1 | 11 | 0.08 |
| | ***QHf.uga-6AL*** | *Xwmc580–Xgwm427* | 6.8** | 155.4 | 13 | 0.09 |

** Significant at the 0.01 probability level

[a] $R^2$, phenotypic variation associated with the QTL

[b] Positive value indicated the allele was inherited from 26R61, and negative value indicated the allele was from AGS 2000

[c] Stable QTL identified in all the environments was in bold

[d] Stable QTL with highly significant LOD value for resistance only to biotype *vH13* was underline

[e] QTL of high LOD value responsible for the tolerance against biotype *vH13* was indicated in bold

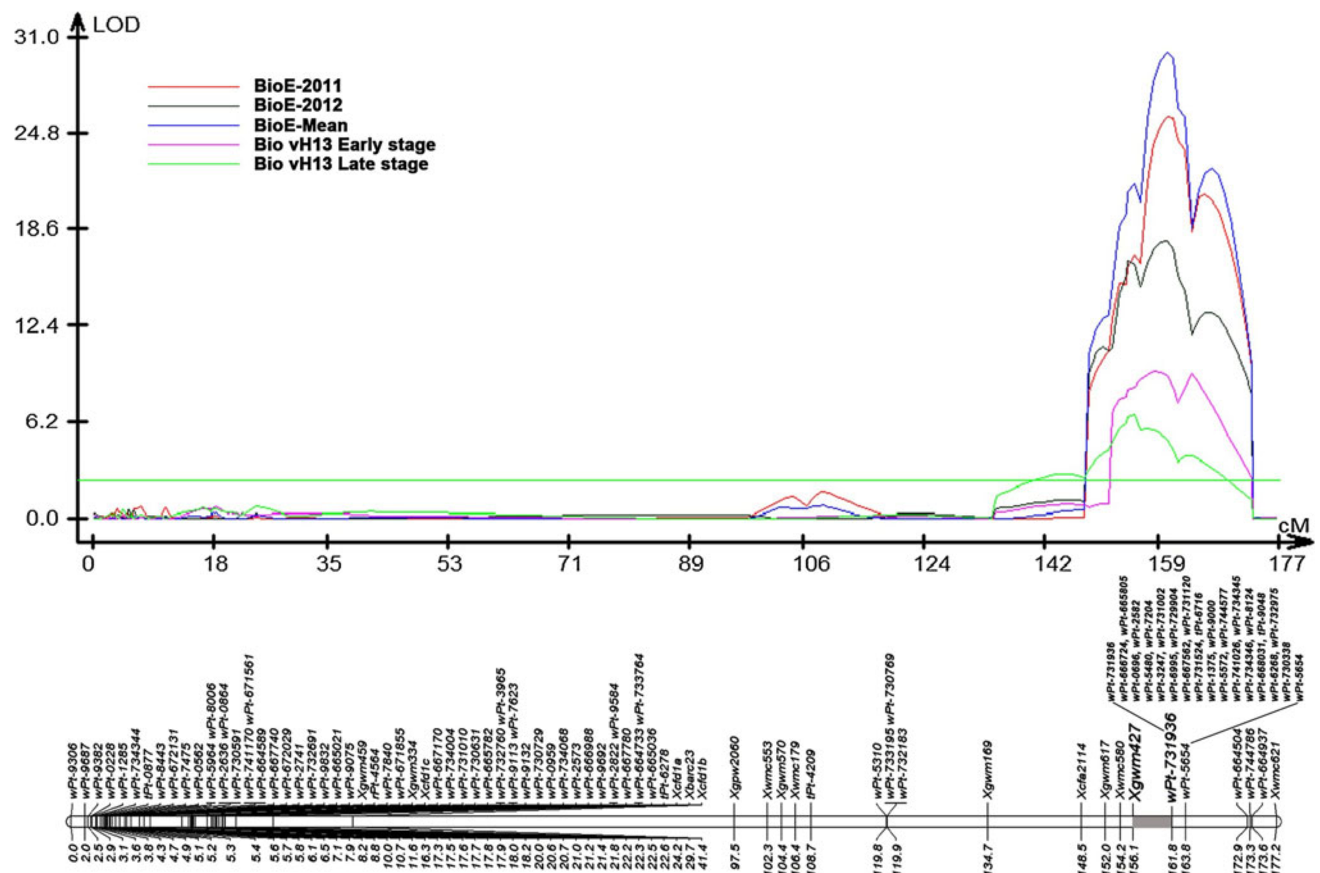


**Fig. 2** *QHf.uga-6AL* of major effect identified across all environments for resistance to both biotypes E and *vH13*; the QTL region is indicated by *gray rectangle*

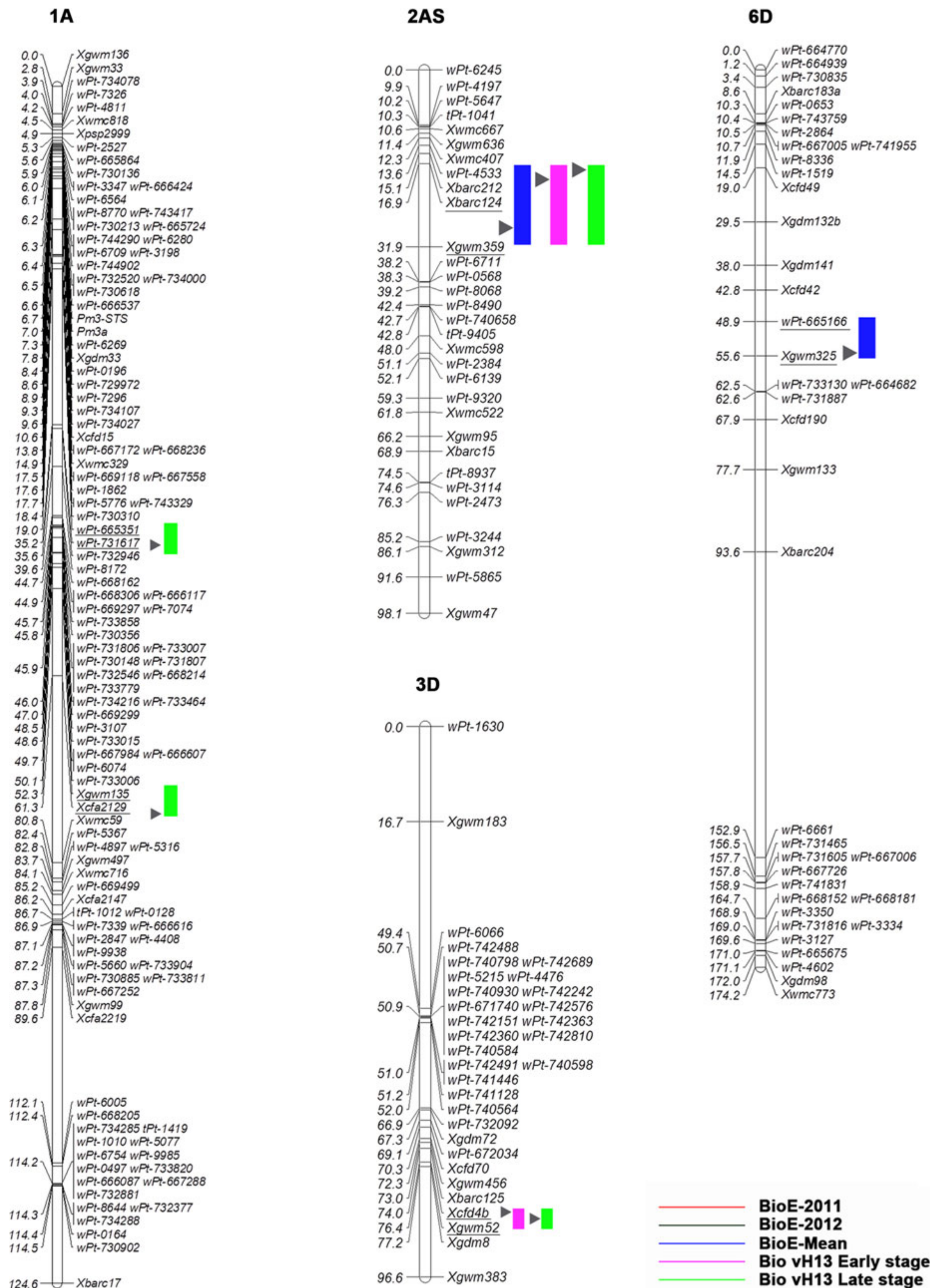


**Fig. 3** Genetic maps of the QTL for resistance to either biotype E or *vH13* in PR61/A2000 population; the *gray triangle* indicates the peak location of each QTL and the flanking markers are *underlined*

resistant to biotype E and O, but currently we are uncertain about its reaction to biotype *vH13*. Because *H1H2* in Dawson, *H7H8* in Seneca, and *HR61* in 26R61 all have very similar reactions to different biotypes, more extensive studies are needed to elucidate their detailed relationships before we can assign an official designation to *HR61*.

Interestingly, *HR61* was not only resistant to biotype E, but also was responsible for the resistance to biotype *vH13*. Its contribution tended to decrease ($R^2$, from 21 to 13 %; LOD, from 9.5 to 6.8) and *QHf.uga-3DL*, in contrast, tended to slightly increase ($R^2$, from 9 to 11 %; LOD, from 5.1 to 5.8) with plant development. With *QHf.uga-3DL* being physically assigned to 3DL2-0.27–0.81 and proximal to the known genes *H24*/*H26*/*H32* on 3DL (Sardesai et al. 2005; Yu et al. 2009), and *QHf.uga-1AL* being assigned to 1AL1-0.17–0.61 where no gene has ever been reported, both QTL should represent new loci for Hessian fly resistance and play particularly important roles in the late seedling stage. It was unexpected that *QHf.uga-6DS-C* and *QHf.uga-1AS*, which had minor effects, were identified from the susceptible parent AGS 2000. They occupy different loci from the known genes *H13* on 6DS and *H9* (*H10*, *H11*, *H16*, *H17* and *Hdic* etc.) gene cluster on 1AS, respectively (Kong et al. 2008; Liu et al. 2005a, b, c), and may also be new components of resistance to Hessian fly. Although these QTL have a minor effect for the current biotypes, it is possible they may have a major effect on new biotype(s) and also help us to understand field resistance or tolerance to Hessian fly in AGS 2000.

Tolerance is somewhat less evident than the major *R* gene resulting from antibiosis and is usually controlled by polygenic factors of small effects, but is still of considerable importance thereby allowing plants to withstand or recover from the injurious effects of Hessian fly attack (Painter et al. 1940). Tolerance to Hessian fly injury is usually associated with characteristics of greater leaf growth, repairing injured parts of the plants or ability to tiller after infestation (Gallun 1972). Early studies reported the tolerance to Hessian fly existed in diverse wheat cultivars in the USA, such as in 'Marquillo' (Painter et al. 1940), 'Kawvale' and its derivatives (Painter and Jones 1945), 'W38' and 'Arthur' (Caldwell et al. 1946; Patterson et al. 1974), but very few genetic studies have been conducted on the inheritance of the tolerance mainly due to its genetic complexity. In the present research, we found three loci typically related to the tolerance response via whole genome mapping and QTL analysis of the PR61/A2000 population. Clearly *QHf.uga-1AL* should be given more credit because it has highly significant LOD value and is detectable only in the late seedling stage when tolerance was evident. Along with *QHf.uga-3DL* and *QHf.uga-1AS*, the three loci represent important components of the total genetic factors of the tolerance, and the closely linked markers will be of assistance to actually breed and select for this tolerance in breeding programs. Importantly, the strategy used in the present research will offer a good starting point for the discovery and mapping of more tolerance genes for resistance to Hessian fly in common wheat.

Biotype L, the most virulent Hessian fly biotype so far, is currently predominant in the southeastern USA (Johnson et al. 2009; Ratcliffe et al. 2000). The *H13* gene, initially transferred from *Aegilops tauschii* to common wheat, is an excellent source of resistance to use in the region and has been incorporated into several commercial wheat cultivars such as AGS 2010, AGS 2026 (PI 658065), Oglethorpe (PI 657986), etc. (Johnson et al. 2006, 2008; Ratcliffe et al. 2000). It is anticipated that the proportion of *H13* virulent biotype (*vH13*) will increase with the wider utilization of the *H13* gene, and actually it has already been observed in some Hessian fly populations from regions of Alabama, Georgia, and South Carolina (Cambron et al. 2010). The cultivar 26R61 has shown good resistance to biotype *vH13* in the present research, and will be useful in combination with *H13* for providing resistance to the currently known Hessian fly biotypes in the southeastern USA. Undoubtedly, the closely linked markers with the resistance gene or QTL in the present study will be of value in selecting or pyramiding of these loci in breeding programs.

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